

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:

YU *et al.*

Appl. No. 09/314,889

Filed: May 19, 1999

For: **Death Domain Containing  
Receptors**

Art Unit: 1646

Examiner: Ulm, J.

Ally. Docket: 1488.0310006/EKS/SGW

**Declaration for Deposited Biological Materials**

Commissioner for Patents  
Washington, D.C. 20231

Sir:

The undersigned attorney of record states as follows:

1. A plasmid containing human cDNA encoding DR3-V1 protein was deposited under the terms of the Budapest Treaty on October 10, 1996. The deposit was made at the American Type Culture Collection (ATCC), 10801 University Boulevard, Manassas, Virginia 20110-2209 (current address), and given accession number 97757. A copy of the ATCC deposit receipt reciting the term of the deposit, as well as the statement of viability, is attached hereto.
2. Assurance is hereby given that all restrictions on the availability to the public of the deposited plasmid referred to above will be irrevocably removed upon the granting of a patent issuing from the patent application captioned above.

DECEMBER 4, 2000

Date



Jonathan L. Klein  
Attorney for Applicant  
Registration No. 41,119

Human Genome Sciences, Inc.  
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Rockville, MD 20850  
Telephone: (301) 251-6015



# American Type Culture Collection

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## BUDAPEST TREATY ON THE INTERNATIONAL RECOGNITION OF THE DEPOSIT OF MICROORGANISMS FOR THE PURPOSES OF PATENT PROCEDURE

### INTERNATIONAL FORM

RECEIPT IN THE CASE OF AN ORIGINAL DEPOSIT ISSUED PURSUANT TO RULE 7.3  
AND VIABILITY STATEMENT ISSUED PURSUANT TO RULE 10.2

To: (Name and Address of Depositor or Attorney)

Human Genome Sciences, Inc.  
Attn: Robert H. Benson  
9410 Key West Avenue  
Rockville, MD 20850

MAR 13 1996  
HGS PATENT DEPT.

Deposited on Behalf of: Human Genome Sciences, Inc.

Identification Reference by Depositor:

ATCC Designation

DNA Plasmid, 231556

97456 - .

The deposits were accompanied by:    a scientific description    a proposed taxonomic description indicated above.

The deposits were received March 1, 1996 by this International Depository Authority and have been accepted.

#### AT YOUR REQUEST:

We will inform you of requests for the strains for 30 years.

The strains will be made available if a patent office signatory to the Budapest Treaty certifies one's right to receive, or if a U.S. Patent is issued citing the strains, and ATCC is instructed by the United States Patent & Trademark Office or the depositor to release said strains.

If the cultures should die or be destroyed during the effective term of the deposit, it shall be your responsibility to replace them with living cultures of the same.

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The viability of the cultures cited above was tested March 7, 1996. On that date, the cultures were viable.

International Depository Authority: American Type Culture Collection, Rockville, Md. 20852 USA

Signature of person having authority to represent ATCC:

Barbara M. Hailey  
Barbara M. Hailey, Administrator, Patent Depository

Date: March 8, 1996



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DNA Plasmid, 1198782 (Docket PF267P1.SKB)

97757

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The viability of the cultures cited above was tested October 17, 1996. On that date, the cultures were viable.

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Signature of person having authority to represent ATCC:

  
Barbara M. Hailey, Administrator, Patent Depository

Date: October 19, 1996

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# IN SITU HYBRIDIZATION AND IMMUNOHISTOCHEMISTRY

14

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*continued*

14.0.1

**SDS electrophoresis buffer, 5×**

15.1 g Tris base  
72.0 g glycine  
5.0 g SDS  
H<sub>2</sub>O to 1000 ml  
Dilute to 1× or 2× for working solution, as appropriate

*Do not adjust the pH of the stock solution, as the solution is pH 8.3 when diluted. Store at 0° to 4°C until use (up to 1 month).*

**SED (standard enzyme diluent)**

20 mM Tris·Cl, pH 7.5  
500 µg/ml bovine serum albumin (Pentax Fraction V)  
10 mM 2-mercaptoethanol  
Store up to 1 month at 4°C

**Sodium acetate, 3 M**

Dissolve 408 g sodium acetate·3H<sub>2</sub>O in 800 ml H<sub>2</sub>O  
Add H<sub>2</sub>O to 1 liter  
Adjust pH to 4.8 or 5.2 (as desired) with 3 M acetic acid

**Sodium acetate buffer, 0.1 M**

*Solution A:* 11.55 ml glacial acetic acid/liter (0.2 M).  
*Solution B:* 27.2 g sodium acetate (NaC<sub>2</sub>H<sub>3</sub>O<sub>2</sub>·3H<sub>2</sub>O)/liter (0.2 M).

Referring to Table A.2.2 for desired pH, mix the indicated volumes of solutions A and B, then dilute with H<sub>2</sub>O to 100 ml. (See Potassium acetate buffer recipe for further details.)

**Sodium phosphate buffer, 0.1 M**

*Solution A:* 27.6 g NaH<sub>2</sub>PO<sub>4</sub>·H<sub>2</sub>O per liter (0.2 M).  
*Solution B:* 53.65 g Na<sub>2</sub>HPO<sub>4</sub>·7H<sub>2</sub>O per liter (0.2 M).

Referring to Table A.2.3 for desired pH, mix the indicated volumes of solutions A and B, then dilute with H<sub>2</sub>O to 200 ml. (See Potassium phosphate buffer recipe for further details.)

**SSC (sodium chloride/sodium citrate), 20×**

3 M NaCl (175 g/liter)  
0.3 M Na<sub>3</sub>citrate·2H<sub>2</sub>O (88 g/liter)  
Adjust pH to 7.0 with 1 M HCl

**STE buffer**

10 mM Tris·Cl, pH 7.5  
10 mM NaCl  
1 mM EDTA, pH 8.0

**TAE (Tris/acetate/EDTA) electrophoresis buffer**

<i>50× stock solution:</i>	<i>Working solution, pH ~8.5:</i>
242 g Tris base	
57.1 ml glacial acetic acid	40 mM Tris·acetate
37.2 g Na <sub>2</sub> EDTA·2H <sub>2</sub> O	2 mM Na <sub>2</sub> EDTA·2H <sub>2</sub> O
H <sub>2</sub> O to 1 liter	

**TBE (Tris/borate/EDTA) electrophoresis buffer**

*10× stock solution, 1 liter:*  
108 g Tris base (890 mM)  
55 g boric acid (890 mM)  
40 ml 0.5 M EDTA, pH 8.0 (20 mM)

**Appendix 2**

**A.2.5**

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*continued*

14.0.1

1c. *Harsh treatment:* Pour several hundred milliliters of boiling 0.1% SDS onto the membrane. Cool to room temperature.

*If a membrane is to be reprobed, it must not be allowed to dry out between hybridization and stripping. If it becomes dry, the probe may bind to the matrix.*

2. Place membrane on a sheet of dry Whatman 3MM filter paper and blot excess liquid with a second sheet. Wrap the membrane in plastic wrap and set up an autoradiograph.

*If signal is still seen after autoradiography, rewash using harsher conditions.*

3. The membrane can now be rehybridized. Alternatively, it can be dried and stored for later use.

*Membranes can be stored dry between Whatman 3MM paper for several months at room temperature. For long-term storage, place the membranes in a desiccator at room temperature or 4°C.*

## REAGENTS AND SOLUTIONS

### *Aqueous prehybridization/hybridization (APH) solution*

5× SSC (APPENDIX 2)

5× Denhardt solution (APPENDIX 2)

1% (w/v) SDS

Add 100 µg/ml denatured salmon sperm DNA (see below) just before use

*Alternatives to Denhardt solution and denatured salmon sperm DNA as blocking agents are listed in Table 2.10.5 (see discussion in critical parameters).*

### *Denatured salmon sperm DNA*

Dissolve 10 mg Sigma type III salmon sperm DNA (sodium salt) in 1 ml water. Pass vigorously through a 17-G needle 20 times to shear the DNA. Place in a boiling water bath for 10 min, then chill. Use immediately or store at –20°C in small aliquots. If stored, reheat to 100°C for 5 min and chill on ice immediately before using.

### *Formamide prehybridization/hybridization (FPH) solution*

5× SSC (APPENDIX 2)

5× Denhardt solution (APPENDIX 2)

50% (w/v) formamide

1% (w/v) SDS

Add 100 µg/ml denatured salmon sperm DNA (see above) just before use

*Alternatives to Denhardt solution and denatured salmon sperm DNA as blocking agents are listed in Table 2.10.5 (see discussion in critical parameters).*

*Commercial formamide is usually satisfactory for use. If the liquid has a yellow color, deionize as follows: add 5 g of mixed-bed ion-exchange resin [e.g., Bio-Rad AG 501-X8 or 501-X8(D) resins] per 100 ml formamide, stir at room temperature for 1 hr, and filter through Whatman no. 1 paper.*

**CAUTION:** Formamide is a teratogen. Handle with care.

### *Labeling buffer*

200 mM Tris·Cl, pH 7.5

30 mM MgCl<sub>2</sub>

10 mM spermidine

### *Mild stripping solution*

5 mM Tris·Cl, pH 8.0

2 mM EDTA

0.1× Denhardt solution (APPENDIX 2)